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A NOTE ON THE MAINTENANCE OF VIRULENCE BY BACILLUS ABORTUS, BANG.*

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In the fall of 1911 the writer brought to this country three cultures of *Bacillus abortus*, Bang, which had been kindly given him by Professors Jensen and Holth of the Veterinary Serum Laboratory in Copenhagen. These cultures were marked as being in the seventh to the ninth generation. I have no notes as to how long these strains had been grown in the laboratory before I received them. However, from various conversations with Dr. Holth I have reason to believe that they had been in the laboratory for more than a year.

As is well known, the Bang bacillus, when first isolated, does not grow readily in the atmospheric pressure of the air. After it has been grown for some generations on culture media it may be induced to grow in the air. These Danish cultures had become accustomed to growing in the air for some time before I received them. Since that time they have never been grown in any other way.

During 1912 these cultures, with others, were repeatedly transferred from agar to agar or from agar to plain peptone broth and then back to agar. It has been found that this latter procedure often revives a somewhat sluggishly growing culture so that it grows much more luxuriantly than before. Thus these Danish cultures had been grown in the laboratory for at least two years and probably longer. During this time they were not passed through animals or in any way handled so as to increase their vigor except the occasional alteration from agar to broth as noted above. It was thought that they had probably lost their virulence, so far as causing abortion in cattle was concerned.

In connection with some experiments on the rate of production of antibodies after inoculation with killed cultures of the abortion bacilli, I inoculated one cow with a mixed culture of these Danish

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strains. These organisms were washed from agar slants with salt solution and one-half of one per cent of carbolic acid added. The mixture was not subjected to heat, as is the usual method, but cultural tests made at that time failed to show any growth. From this it was concluded that the carbolic acid had been sufficient to kill the bacteria.

Since that time I have been able to demonstrate that one-half of one per cent carbolic acid is not sufficient to kill the abortion bacilli. Such treatment appears to retard their growth slightly, but otherwise they are as vigorous as before. I failed, for some reason, to obtain a growth in the test mentioned above, although, as subsequent results showed, the organisms were probably not dead.

The cow used in this experiment was a grade Jersey purchased in July, 1912, for experimental purposes. According to her previous owner she had never aborted and came from a farm where there had been no abortion. She was bred June 10, 1912, i.e., before coming to the Experiment Station.

During the summer and autumn her blood was tested at frequent intervals for abortion antibodies by both the agglutination and complement fixation tests.¹ At no time could abortion antibodies be demonstrated in her blood except as noted in the table below.

On November 25, 1912, she received subcutaneously, 20 c.c. of a dilute suspension of the carbolized but unheated Danish cultures referred to above. This suspension showed a count of approximately 500,000 bacteria to the cubic centimeter.² Thus there were injected in all, about 10,000,000 bacteria, supposedly dead. The history of this cow from just before the injection to the time of writing is given below in tabular form.

Nov. 14, 1912—Blood test gave no reaction.

Nov. 25, 1912—Subcutaneous injection of abortion bacilli as noted above. Blood test gave no reaction.

Dec. 2, 1912—Blood test gave no reaction.

Dec. 9, 1912—Blood test gave slight reaction. Fixation of complement with 0.05 c.c. serum but not with less amounts.

¹ The technic used in these tests has been fully described in a previous paper—*Ky. Agric. Exper. Sta., Ann. Rpt.*, 1912, Bull. 166, p. 303.

² This count was made after the method described by Callison, *Jour. Med. Research.*, 1912, 27, p. 225.

- Dec. 20, 1912—Blood test showed strong reaction—agglutination with 0.001 c.c. of serum. Fixation of complement with 0.005 c.c. serum.
- Jan. 10, 1913—Showed signs of aborting.
- Jan. 13, 1913—Blood test gave strong reaction. Same titre as December 20, 1912.
- Jan. 16, 1913—Aborted a well-developed calf. Large amount of typical yellowish exudate around the cotyledons. Abortion bacilli isolated. (Cf. below.)
- Feb. 10, 1913—Blood test gave strong reaction.

The cow was kept during this period in a barn with other cows where abortion had been very prevalent. Under such a condition she might very easily have become infected from such sources. The following observations, however, do not support this view.

A few hours after the abortion, the afterbirth was removed from the cow by a veterinarian. In this way I was able to obtain a number of cotyledons, showing typical exudate, which had had little chance to become contaminated with other organisms. Material from the foetal stomach and intestines was also obtained free from contamination. Plain agar plates were inoculated from this material. After 24 hours at 37° C. they gave no growth except a very few contaminating colonies from the cotyledons. About half the plates were then placed in a Novy jar with *Bacillus subtilis* after the method of Nowac.¹ On the second day it was noted that the plates remaining in the air developed a fine, opalescent, dew drop growth characteristic of the Bang bacillus. This growth increased within the next day or two so that a very rich growth, and in the majority of plates, a pure culture appeared. By microscopical examination and also by using it as antigen in a complement fixation test with an immune serum, this growth proved to be the *B. abortus*. After four days the plates were removed from the Novy jar and these also had a rich growth of the same organism. However, the growth obtained in the Novy jar under reduced pressure of oxygen was in no respect better than that obtained in the free air. Those who have had experience in isolating this organism from aborted material will realize that it is certainly very unusual to find it growing in the free air in the first generation, at least on the second day of incubation. Fabyan² however, states

¹ *Ann. de l'Inst. Pasteur*, 1908, 22, p. 541; Good, E. S., *Ky. Agric. Exper. Sta.*, 1912, *Bull.* 165, p. 227

² *Jour. Med. Research*, 1912, 26, p. 441.

that sometimes this organism will grow in the air when first isolated. He says: "It is probable that strains will be found to vary more or less in their relation to oxygen and to the kinds of culture media usually employed. . . . For instance, culture II, *a* and *b*, we were able to isolate without *B. subtilis* directly from guinea-pigs inoculated with the original material. In this instance, the tissues of two guinea-pigs were broken up as described, and spread over slants of ordinary agar containing one-half to one cubic centimeter of defibrinated guinea-pigs' blood. After a period of some four days, pure cultures were obtained."

In my own experience which has covered the examination of a considerable number of aborted foeti I have never been able to obtain a growth of this organism on the surface of agar from material direct from an aborting cow or foetus. It will be noted that Fabyan obtained this surface growth after passage through a guinea-pig. Whether this modified the organism in any way I am not able to say. It will further be noted that in the case I have described above I obtained a growth on plain agar (without any serum) and on the second day of incubation. These characteristics correspond very well with those of the Danish strains which were injected. Fabyan¹ also makes the following statement regarding this organism: "It is an interesting fact that after the organism has adapted itself to ordinary aërobic conditions it does not lose this characteristic by further passage through guinea-pigs. Some of our guinea-pigs so inoculated have lived fifteen to twenty weeks and yet upon making cultures from the spleen *B. abortus* developed even at room temperature."

I may further say that the strain of *B. abortus* which is prevalent in this portion of the Experiment Station herd, is very difficult to grow under any condition. I have frequently been unable to grow it at all, although from microscopical examination I was certain that the organism was present in the material. It is also very difficult to accustom this strain to grow in the free air.

From these considerations I think we may reasonably infer that the abortion in this case was due to the injection of the carbolyzed but unheated Danish culture. Thus these organisms which have

¹ *Loc. cit.*

been grown in the laboratory for two years and probably longer, have not lost their virulence so far as causing abortion in cattle is concerned.

We may also note that after the subcutaneous injection we were able to detect abortion antibodies after 14 days but not at the end of 7 days. At the end of 25 days the blood showed its strongest reaction. It has maintained this titre up to the time of writing. The abortion occurred 52 days after the inoculation. These observations agree reasonably well with the data obtained by MacFadyen and Stockman¹ and others with experimentally inoculated cows.

SUMMARY.

The subcutaneous injection of a cow with 20 cubic centimeters of a dilute mixture of three Danish strains of *B. abortus* which had been grown in the laboratory for more than two years, produced a typical abortion 52 days after the injection. The mixture had not been heated but contained one-half of one per cent of carbolic acid. The cultural test made at the time revealed no growth from this mixture. Since that time it has been found that one-half of one per cent of carbolic acid is not sufficient to kill this organism.

Cultures of this organism were isolated from the afterbirth and foetus. These cultures grew readily in the air. Growth appeared on plain agar on the second day of incubation. In these respects they agreed with the Danish strains injected and were totally different from the strain of this organism prevalent in the Experiment Station herd.

Blood tests at various intervals showed the appearance of antibodies 14 days after inoculation or about five weeks before the abortion.

Thus after more than two years' growth under laboratory conditions this organism has retained its original virulence.

¹ *Rpt. of Abortion Committee*, Bd. of Agric. and Fisheries of Great Britain, London, 1909, pt. 1.